PB83-189084

Morphometric, Biochemical, and Physiological Assessment of Perinatally Induced Renal Dysfunction

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1983

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. PB83-139064
EPA-600/J-83-006	JOURNAL ARTICLE	

4. TITLE AND SUBTITLE	5. REPORT DATE
Morphometric, Biochemical, and Physiological Assessment of Perinatally Induced Renal Dysfunction	1983
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Robert J. Kavlock, Jacqueline Gray	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Perinatal Toxicology Branch/Developmental Biology Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711	ACSL1A
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Office of Research and Development Health Effects Research Laboratory US Environmental Protection Agency Research Triangle Park, NC 27711	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

Published In: Journal of Toxicology and Environmental Health, 11:1-13, 1983

16. ABSTRACT

Three chemicals, known either to alter renal development when administered during fetal development or to affect renal function when administered to adult rats, were administered to Sprague-Dawley rats at critical periods of renal development. Chlorambucil (CHL) was administered ip on d 11 of gestation at doses of 0.3. and 6 mg/kg; nitrofen (2,4-dichlorophenyl p-nitrophenyl ether) (NIT) was given po on d 8-16 of gestation at 0.4.17, 12.5, and 25 mg/kg.d and mercuric chloride (MER) was given sc on postnatal d 1 at 0.14, and 28 ug/pup. To assess the effects of these toxicants on the functional development of the kidneys, a diuresis test with and without antidiuretic hormone was applied on postnatal d 3 (PD 3); a hydropenia test on PD 6; and kidney weights, glomerular counts in midhilar cross sections, and the specific activity of renal alkaline phosphatase were determined on PD 3 and 6. Data from pups with obvious malformations of the kidneys was eliminated from the statistical analyses of the data so that emphasis could be placed on alterations of functional development in individuals with apparently morphologically normal kidneys. CHL retarded the growth and biochemical differentiation of the kidney at 6 mg/kg. Pups from this treatment groups showed an attenuated response to exogenously administered antidiuretic hormone. NIT impaired growth and altered renal morphology at a dose of 12.5 mg/kg.d and altered physiological responses in the absence of anatomical changes at a dose of 4.17 mg/kg.d. MER, at doses near the maximum tolerated, failure to alter any parameter, indicating that the very young animal differs markedly from the adult in response to that compound. The data indicate that relatively simple tests of renal function are useful in the detection of perinatally induced nephrotoxicity.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
Release to Public	Unclassified	14
	20. SECURITY CLASS (This page)	22. PRICE
	Unclassified	

EPA-600/J-83-006
JOURNAL ARTICLE

MORPHOMETRIC, BIOCHEMICAL, AND PHYSIOLOGICAL ASSESSMENT OF PERINATALLY INDUCED RENAL DYSFUNCTION

Robert J. Kavlock, Jacqueline A. Gray

Perinatal Toxicology Branch, Developmental Biology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina

Three chemicals, known either to alter renal development when administered during fetal development or to affect renal function when administered to adult rats, were administered to Sprague-Dawley rats at critical periods of renal development. Chlorambucil (CHL) was administered ip on d 11 of gestation at doses of 0, 3, and 6 mg/kg; nitrofen (2,4-dichlorophenyl p-nitrophenyl ether) (NIT) was given po on d 8–16 of gestation at 0, 4.17, 12.5, and 25 mg/kg•d; and mercuric chloride (MER) was given sc on postnatal d 1 at 0, 14, and 28 μg/pup. To assess the effects of these toxicants on the functional development of the kidneys, a diuresis test with and without antidiuretic hormone was applied on postnatal d 3 (PD 3); a hydropenia test on PD 6; and kidney weights, glomerular counts in midhilar cross sections, and the specific activity of renal alkaline phosphatase were determined on PD 3 and 6. Data from pups with obvious malformations of the kidneys was eliminated from the statistical analyses of the data so that emphasis could be placed on alterations of functional development in individuals with apparently morphologically normal kidneys. CHL retarded the growth and biochemical differentiation of the kidney at 6 mg/kg. Pups from this treatment groups showed an attenuated response to exogenously administered antidiuretic hormone. NIT impaired growth and altered renal morphology at a dose of 12.5 mg/kg•d and altered physiological responses in the absence of anatomical changes at a dose of 4.17 mg/kg•d. MER, at doses near the maximum tolerated, failed to alter any parameter, indicating that the very young animal differs markedly from the adult in response to that compound. The data indicate that relatively simple tests of renal function are useful in the detection of perinatally induced nephrotoxicity.

INTRODUCTION

With increased emphasis being placed on the detection of functional abnormalities in teratology studies, the desirability of validated methods to assess organ function in neonatal rodents is obvious. While techniques for the evaluation of renal dysfunction in adult rodents are well established (Berndt, 1976), little attention has been paid to such assessments in the

Requests for reprints should be sent to R. J. Kavlock, U.S. Environmental Protection Agency, Developmental Biology Division/MD-67, Research Triangle Park, North Carolina 27711.

Journal of Toxicology and Environmental Health, 11:1–13, 1983
Hemisphere Publishing Corporation

neonatal age class. Zeman and co-workers (Zeman, 1968; Hall and Zeman, 1968; Allen and Zeman, 1973), working with maternal protein restriction, were among the first to publish on physiologic alterations in renal development subsequent to an *in utero* insult. More recent studies have examined the effect of maternal administration of methyl salicylate on the development of the renal papilla (Woo and Hoar, 1972), the effect of *in utero* exposure to dinoseb, paraquat, and TCDD on the uptake of organic anions and phlorizin binding capacity by renal tissue slices (Gibson, 1976), the age-related effects of potassium dichromate and glycerol on PAH excretion (Braunlich et al., 1979), and the effects of postnatal lead exposure on a variety of functional parameters (Johnson and Kleinman, 1979; Johnson et al., 1980), although the later study examined only postweanling animals.

Recently, we developed a series of morphological, biochemical, and physiological tests applicable to the assessment of renal function in neonatal rat pups and have described the normative profile of development for those parameters (Kavlock and Gray, 1982). This present report details the use of these tests in the detection of chemically induced renal dysfunction in the neonate. Two of the compounds selected (chlorambucil and nitrofen) have been reported to produce abnormalities of urogenital system when administered during gestation (Monie, 1961; Costlow and Manson, 1981); in addition, we have previously shown (Kavlock et al., 1982) that fetuses exposed to these compounds showed delayed morphological and biochemical differentiation of the kidneys. We were thus interested in examining the postnatal sequelae of the prenatal retardation, as well as whether renal dysfunction could be detected in siblings of pups that did not display overt malformations of the urogenital tract. The third compound we selected was mercuric chloride, which has been used extensively by other investigators studying the effects of chemically induced renal dysfunction in adult animals (McDowell et al., 1976; Zalme et al., 1976).

METHODS

Animals

Ninety-day-old, time pregnant, Sprague-Dawley rats were obtained from Charles River Breeding Laboratory, Wilmington, Mass. The day that sperm was found in the vaginal smear was considered d 1 of pregnancy. Animals were housed individually in plastic cages on pine shaving bedding. Food (Purina Rat Chow, Ralston-Purina, St. Louis, Mo.) and water were provided *ad libitum*. Temperature was maintained at 20–24°C, relative humidity at 40–50%, and the diurnal cycle was 6 a.m. to 6 p.m. EST. In our laboratory, this strain normally delivers the evening of d 22 of gestation, and the following day was considered d 1 of postnatal life (PD

1). When a compound was administered directly to postnatal animals (as was the case with mercuric chloride), litter sizes on PD 1 were held to a maximum of 10 pups (5 ± 1 of each sex) to minimize effects of varying litter size on growth. For compounds administered during gestation (chlorambucil and nitrofen), litter sizes at birth were not controlled; rather, the litters were examined daily for evidence of postnatal mortality. Dead or moribund pups were retrieved from the nest and necropsied to characterize any morphological lesions related to exposure.

Treatment Regimens

Chlorambucil (CHL) was dissolved in corn oil and administered ip on d 11 of gestation as described by Monie (1961). Doses were 0, 3, and 6 mg/kg·d. Nitrofen (NIT) was dissolved in corn oil and administered via oral intubation on gestational d 8–16 as described by Kimbrough et al. (1974). Doses were 0, 4.17, 12.5, or 25.0 mg/kg·d. Mercuric chloride was dissolved in sterile saline and administered sc at doses of 28, 14, or 0 μg/pup on PD 1. These dosages were equivalent to 4 and 2 mg/kg, respectively and represent an adult nephrotoxic dose. The injection volume was 20 μl.

Renal Function Tests

Procedures used to examine functional capacity of the neonatal rat kidney have been described previously (Kavlock and Gray, 1982). Pups that were runted or were obviously in poor health (e.g., those that lacked milk bands) were not utilized in the function tests. Otherwise, individuals were randomly selected from among the litters in the various treatment groups for testing. Briefly, these tests include water diuresis and anti-diuretic hormone tests applied on PD 3 and a hydropenia test applied on PD 6. In the diuresis tests, two pairs of male and female pups from each of five litters were removed from the nest and their bladders were voided by gentle perianal stimulation. Pups in the first pair received an intragastric load of deionized distilled water equal to 5% of their body weight. Pups in the second pair received the water load plus a subcutaneous injection of an analog of lysine vasopressin that lacks pressor activity [desmopressin acetate (DDAVP), Ferring Pharmaceuticals, Inc.] at a dosage of 100 mU/kg. During the course of the test the pups were isolated from the nest and maintained in beakers at 32°C. At 40 and 80 min after receiving the load, the urine produced was collected and quantified. Results are expressed as the percent of the water load excreted per interval for pups receiving only water, and as the difference between the percent excreted by the water-loaded pups and the percent excreted by the pups who also received DDAVP.

Those pups that had received the water load but no DDAVP on PD 3 were marked, returned to the nest, and used for the hydropenic response on PD 6. To initiate this test, their bladders were voided and an aliquot of urine saved. The pups were then isolated in warmed beakers. At 3 h later, the bladders were voided and the urine discarded. At 6 h after removal

from the nest, the bladders were again voided and an aliquot of urine was saved to assess the ability to concentrate urine. The osmotic pressure of an 8-μl sample of each aliquot was then determined using a vapor-pressure osmometer (Model 5001B, Wescor, Inc., Logan, Utah). The difference between the osmotic pressure collected during hydropenia (the 6-h urine sample) was then compared with the osmotic pressure of urine in the basal state (the initial urine sample).

The pups used in the hormone response study on PD 3 and those used in the hydropenia study on PD 6 were decapitated at the conclusion of their test and the kidneys were removed, weighed, and preserved for further study. The left kidney was fixed in a solution of 5% formaldehyde, 5% glacial acetic acid, and 70% ethanol, embedded in paraffin, and 8 μm cross sections were taken 60 μm apart through the mid-hilar region. The sections were then stained with hematoxylin and eosin, and the fully formed glomeruli were counted. The right kidney was frozen in a dry ice/acetone bath and stored in plastic vials at −60°C. Later, the kidney was thawed, homogenized in a 2-ml Potter-Elvehjem tissue homogenizer in pH 7.4 Hepes buffer (5% w/v homogenate), centrifuged at 5000 X g for 15 min in a table-top centrifuge (Model CL, International Equipment Co.), and assayed for alkaline phosphatase activity—a marker enzyme of development of the brush border of renal tubules (Washstein, 1955)—using Sigma 104 Phosphatase Substrate (Sigma Chemical Co.) and protein by the Peterson modification of the Lowry technique (Peterson, 1977). Bovine serum albumin (Sigma Chemical Co.) was used as the protein standard. Alkaline phosphatase activity was expressed in units (μmol *p*-nitrophenol produced per hour).

Statistical Analysis

The data were analyzed using analysis of variance techniques available in the Statistical Analysis System (SAS, 1979). Age and dose were used as main effects for the variables of body weight, kidney weight, glomerular counts, total protein, and total alkaline phosphatase activity. A log transformation was used to correct for heterogeneity of variance for these variables. When no significant age-by-dose interaction was evident, the variables were pooled across doses for simplicity of interpretation. Time and dose were the independent variables for the water diuresis and hormone response study, and dose was the independent variable in the hydropenia study. All paired comparisons were made using Student's *t*-test on the least-squares means. All effects are reported significant at the $p < 0.05$ level.

RESULTS

General

Toxic effects observed in the immediate neonatal period are summarized in Table 1. CHL was teratogenic at 6 mg/kg, producing

TABLE 1. Summary of Toxicity Observed in Litters Prior to Initiation of Renal Tests (Mean ± SE)

Dose (mg/kg)	Number of litters	Total born	Percent surviving to d 2	Comments
			Chlorambucil	
0	9	10.6 ± 1.4	97 ± 2	
3	8	11.1 ± 1.1	100 ± 0	
6	10	9.7 ± 1.0	41 ± 13[b]	Of 35 dying pups recovered from the nest, 32 had short and/or kinky tails, 24 had posterior oligodactyly, 15 had diaphragmatic hernias, 6 had renal agenesis, and 4 each had hydronephrosis and hydroureter.
			Nitrofen	
0	18	10.8 ± 0.6	99 ± 1	
4.17	10	9.1 ± 1.1	99 ± 3	Of 3 dying pups recovered from the nest, 2 had diaphragmatic hernias.
12.5	14	9.9 ± 0.8	69 ± 7[b]	Of 35 dying pups recovered from the nest, 35 had diaphragmatic hernias.
25.0	8	8.4 ± 1.5	37 ± 12[b]	Of 32 dying pups recovered from the nest, 19 had diaphragmatic hernias.
			Mercuric chloride[a]	
0	6	9.8 ± 0.2	100 ± 0	
2	6	10.0 ± 0	100 ± 0	
4	6	9.8 ± 0.2	98 ± 2	

[a] Litter size was limited to a maximum of 10 pups on the day of birth (see methods).
[b] Significantly different from control value, $p < 0.05$.

primarily diaphragmatic hernias and reduction anomalies of the posterior portion of the body (short and/or kinky tails, oligodactyly, renal agenesis). Of the pups born to dams in that dose group, 60% failed to survive 24 h. Less severe reduction anomalies, involving the tail and the hind limbs, were observed in half of the pups surviving 6 mg/kg. No decreased survival or externally visible malformations were seen in pups born to dams receiving 3 mg/kg. NIT reduced pup viability (primarily caused by diaphragmatic hernias) at doses of 12.5 and 25 mg/kg·d. Surviving pups had no externally visible defects. No signs of toxicity were observed in pups receiving injections of MER of up to 4 mg/kg. Two pups from the 6-mg CHL/kg group and one pup each from the 12.5- and 25-mg NIT/kg groups were determined by histological examination to be markedly hydronephrotic, and data derived from those pups were eliminated from all statistical analyses. The performance of the individuals in the function tests was uniformly poor and is noted at the conclusion of this section.

Morphometry and Biochemistry

The effects of administration of CHL, NIT, and MER on morphology and biochemistry are presented in Table 2. For ease of interpretation, the

TABLE 2. Morphometric and Biochemical Effects Observed in 3- and 6-d-old Rat Pups[a]

Dose (mg/kg)	Body weight (g)			Kidney weight (mg)			Glomerular counts			Protein content (μg)			Alkaline phosphatase (activity)		
	PD 3	PD 6	Pooled	PD 3	PD 6	Pooled	PD 3	PD 6	Pooled	PD 3	PD 6	Pooled	PD 3	PD 6	Pooled
								Chlorambucil							
0	9.1 ± 0.2	14.3 ± 0.8	11.3	62 ± 3	105 ± 5	80	—	183 ± 7		1010 ± 58	2009 ± 134	1394	216 ± 15	272 ± 15	237
3	9.4 ± 0.1	13.8 ± 0.3	11.3	61 ± 3	103 ± 3	78	—	197 ± 7		977 ± 43	1978 ± 65	1380	230 ± 10	312 ± 12	265
6	8.3 ± 0.5	14.1 ± 0.6	10.8	48 ± 3	97 ± 4	68[b]		172 ± 13		761 ± 40	1857 ± 100	1176[b]	191 ± 13	326 ± 32	244
								Nitrofen							
0	9.4 ± 0.2	15.1 ± 0.2	11.8	70 ± 2	103 ± 3	84	133 ± 3	200 ± 6	162	950 ± 40	1834 ± 102	1284	280 ± 12	280 ± 12	258
4.17	9.1 ± 0.2	15.2 ± 0.5	11.7	65 ± 2	93 ± 5	76	145 ± 6	176 ± 12	157	826 ± 31	1907 ± 216	1217	266 ± 16	318 ± 28	283
12.5	8.6 ± 0.3	13.3 ± 0.5	10.5[b]	60 ± 2	90 ± 3	72[b]	130 ± 4	181 ± 4	153[b]	798 ± 48	1563 ± 85	1078[b]	237 ± 18	292 ± 13	251
25.0	7.0 ± 0.6	11.1 ± 1.5	8.5[b]	49 ± 6	88 ± 12	62[b]	103 ± 6	162 ± 12	123[b]	832 ± 76	1569 ± 160	1103[b]	176 ± 31	269 ± 27	201[b]
								Mercuric chloride							
0	9.7 ± 0.2	14.7 ± 0.4	11.8	72 ± 2	109 ± 3	89	145 ± 6	221 ± 10	178	966 ± 34	1511 ± 59	1192	263 ± 10	358 ± 22	298
2	9.4 ± 0.1	14.7 ± 0.3	11.7	69 ± 2	108 ± 3	86	137 ± 4	227 ± 8	175	912 ± 52	1459 ± 68	1141	292 ± 14	371 ± 13	327
4	9.5 ± 0.3	14.5 ± 0.4	11.7	71 ± 2	106 ± 4	86	136 ± 6	212 ± 9	168	980 ± 55	1580 ± 112	1224	259 ± 10	385 ± 18	313

[a] See Table 3 for the numbers of pups used on PD 3, and Table 4 for the numbers used on PD 6.
[b] Significantly different from control value, $p < 0.05$.

parameters were pooled across doses when no significant age-by-dose interactions were found. All five parameters showed highly significant changes between PD 3 and 6. Between PD 3 and 6, control body and kidney weights increased approximately 50%, total renal protein content nearly doubled, while alkaline phosphatase activity increased about 40% and the number of glomeruli in a mid-hilar cross section increased by about 50%. Kidney weight and renal protein content were reduced in pups in 6 CHL/kg group. These effects occurred without a significant overall effect on body weight. Body weight, kidney weight, renal protein content, and glomerular counts were significantly affected by 12.5 and 25.0 mg NIT/kg. Renal alkaline phosphatase activity was affected by 25.0 mg NIT/kg.

Water Diuresis

The percentage of the water load excreted within 80 min after intragastric administration is presented in Table 3. Control pups excreted between 50 and 60% of the load during the 80-min observation period. Total urine production was suppressed by 12.5 and 25 mg NIT/kg. When the total volume of excretion was affected, effects were always evident in

TABLE 3. Effects of a Water Load Equal to 5% of the Body Weight on Urine Formation in 3-d-old Pups with and without Antidiuretic Hormone

Dose (mg/kg)	Water diuresis[a]			DDAVP response[b]		
	0–40 min	40–80 min	Total	0–40 min	40–80 min	Total
			Chlorambucil			
0	26 ± 3 (10)[c]	30 ± 2	56 ± 3	−15 ± 2 (10)	−25 ± 1	−40 ± 2
3	32 ± 4 (10)	33 ± 2	65 ± 4	−13 ± 4 (10)	−26 ± 1	−40 ± 5
6	19 ± 4 (8)	27 ± 2	46 ± 5	−10 ± 1 (10)	−19 ± 3[d]	−29 ± 3[d]
			Nitrofen			
0	31 ± 2 (20)	28 ± 2	60 ± 3	−18 ± 2 (20)	−22 ± 1	−40 ± 3
4.17	29 ± 3 (10)	30 ± 2	59 ± 4	−17 ± 2 (10)	−25 ± 1	−42 ± 3
12.5	21 ± 2 (19)[d]	26 ± 2	47 ± 4[d]	−8 ± 2 (20)[d]	−20 ± 1	−28 ± 2[d]
25	15 ± 3 (10)[d]	19 ± 5[d]	34 ± 8[d]	−9 ± 1 (8)[d]	−12 ± 3[d]	−21 ± 4[d]
			Mercuric chloride			
0	23 ± 3 (10)	29 ± 2	53 ± 3	−8 ± 5 (10)	−26 ± 1	−34 ± 6
2	20 ± 3 (10)	30 ± 1	50 ± 3	−8 ± 3 (10)	−24 ± 1	−32 ± 3
4	25 ± 3 (10)	27 ± 2	52 ± 3	−9 ± 2 (10)	−20 ± 1	−29 ± 2

[a] Data presented as the percent of the administered load excreted per time interval.
[b] Data presented as the difference in percent excretion between pups receiving the water load and those receiving the water load plus DDAVP.
[c] Mean plus or minus standard error of the mean (number of individuals tested).
[d] Significantly different from control value, $p < 0.05$.

the 0–40-min time period. The diminution in excretion was less evident between 40 and 80 min after loading, as 12.5 mg NIT/kg did not significantly depress urine formation in that period. As the results of a water diuresis test could be affected by basal state of hydration of the subject, it should be noted that pups in the 25 mg NIT/kg group had significantly elevated basal urinary osmolalities (see below), an indicator of a state of dehydration. Such a condition would promote retention of a water load. However, pups in the 12.5 mg NIT/kg group did not show similar evidence of being dehydrated, and the diminution in response to water load in this dose group represents a true functional deficit. Administration of up to 6 CHL/kg or 4 mg MER/kg did not affect the ability of neonates to excrete a water load.

DDAVP Response

The percentage of a water load excreted within 80 min in the presence of antidiuretic hormone relative to that produced without the hormone is presented in Table 3. In control hormone-injected pups, approximately 20% of the water load was excreted during the 80-min observation period as compared to approximately 60% for pups not receiving the hormone. Thus, urine production during a water-forced diuresis was reduced approximately 40% by the presence of the hormone. Pups in the groups receiving 6 mg CHL/kg and 12.5 and 25 mg NIT/kg showed an attenuated response to antidiuretic hormone (i.e., pups in those groups excreted relatively greater amounts of urine). The results of an attenuated response to exogenous antidiuretic hormone in hydrated subjects indicates failure of either the renal collecting ducts to respond to the hormone or the ability of the renal medulla to maintain the concentration gradient necessary to promote free-water retention. This effect is noted even in the apparently dehydrated 25-mg NIT/kg pups (see results of hydropenia response). Such a condition should have promoted rather than reduced retention of the water load, and therefore the significance of the effect in terms of renal functional impairment is highly significant. Pups exposed to MER responded to administration of DDAVP in a manner similar to their concurrent controls.

Hydropenia Response

The basal (those recorded immediately upon removal from the nest) and hydropenic (those recorded following 6 h of fluid deprivation) urinary osmotic pressures of 6-d-old rat pups are presented in Table 4. Basal urinary osmotic pressures averaged between 319 and 432 mOs*M* for control pups. Hydropenic urine osmotic pressures were generally 300–400 mOs*M* greater than basal pressures for control groups. Pups in the 4.17- and 12.5-mg NIT/kg groups failed to concentrate their urine to the same extent as control pups, as evidenced by their significantly lower hydropenic urinary osmolalities. The overall change in urine osmotic pressure

TABLE 4. Urine Concentrating Ability Recorded in 6-d-old Rat Pups following 6 h of Fluid Deprivation

Dose (mg/kg)	Basal urine osmolality (mOs*M*)	Hydropenic urine osmolality (mOs*M*)	Osmolality increase (mOs*M*)
	Chlorambucil		
0	432 ± 34 (10)[a]	670 ± 17	237 ± 44
3	431 ± 23 (10)	662 ± 10	231 ± 26
6	354 ± 38 (6)	682 ± 15	328 ± 46
	Nitrofen		
0	317 ± 22 (22)	726 ± 12	410 ± 27
4.17	293 ± 24 (10)	666 ± 19[b]	373 ± 32
12.5	383 ± 27 (20)	657 ± 13[b]	274 ± 29[b]
25.0	616 ± 75 (7)[b]	712 ± 22	96 ± 59[b]
	Mercuric chloride		
0	325 ± 65 (10)	730 ± 21	406 ± 75
2	341 ± 19 (10)	680 ± 18	339 ± 28
3	302 ± 40 (10)	651 ± 36	349 ± 59

[a]Mean plus or minus standard error of the mean (number of individuals tested).
[b]Significantly different from control value, $p < 0.05$.

occurring in response to 6 h of fluid deprivation was significantly lower in the groups receiving 12.5 and 25 mg NIT/kg. Pups in the 25-mg NIT/kg group were apparently dehydrated at the start of concentration test, as their basal osmotic pressures averaged 616 mOs*M*. As discussed above, this effect diminishes the significance of the reduced response to a water load by pups in this group, but enhances the significance of the reduced response to DDAVP administration. While a trend was observed for a diminished concentrating response in MER treated pups, no significant changes were observed for that compound.

Hydronephrotic Animals

Two pups from the 6-mg CHL/kg group and one pup each from the 12.5- and 25-mg NIT/kg groups were determined by histological examination to be markedly hydronephrotic. The hydronephrotic CHL-treated pups weighed an average of 30% less than nonhydronephrotic pups from the same treatment groups and had kidneys that weighed 35% less and contained 40% less protein and 35% less alkaline phosphatase activity. Hydronephrotic NIT-treated pups were similarly retarded in overall growth compared to non-hydronephrotic-treated pups, but kidney weights were heavier than in other pups. The greater organ weights were due to large quantities of fluid trapped in the pelvis region, as renal protein content

and alkaline phosphatase activity were reduced. Histologically, the severity of the hydronephrotic lesion was greater in the NIT-treated pups than in the CHL-treated pups. The presence of hydronephrosis greatly diminished the ability to excrete a water load. The two affected CHL pups excreted only 27 and 16% of their respective water load (versus a treatment mean of 46% and a concurrent control mean of 56%), while no urine could be collected from the hydronephrotic 25-mg NIT/kg pup during the 80-min observation period. The other affected NIT-treated pup (from the 12.5-mg/kg group) received the water load and DDAVP and also failed to produce any urine during the observation period. In the renal concentrating ability tests, the hydronephrotic CHL-treated pups had the two lowest osmotic pressure increases (77 and 234 mOs*M*) of any pups in that dosage group, while the hydronephrotic NIT-treated ones obtained a renal osmotic pressure of only 373 mOs*M*, far below the group average of 715 mOs*M*.

DISCUSSION

In a previous experiment (Kavlock et al., 1982) we demonstrated that exposure during gestation to fetotoxic agents did not affect all organs equally. Rather, for the compounds we studied, the kidneys and liver appeared more sensitive to deficits in morphological and biochemical development than were the brain and lungs. In this experiment we attempted to determine for two of those compounds (chlorambucil and nitrofen) whether postnatal functional disturbances could be detected in the kidneys of postnatal animals. We also attempted to include in this experiment a "positive control" to monitor the sensitivity of the various tests we were utilizing. For this reason, neonatal pups were exposed to doses of mercuric chloride known to induce acute renal failure in adult rats (McDowell et al., 1976; Zalme et al., 1976).

Prenatal administration of both chlorambucil and nitrofen resulted in expected malformations of the kidneys (Monie, 1961; Costlow and Manson, 1981). It should be noted that our incidence of hydronephrosis after nitrofen administration was lower than that observed previously, probably caused by differences in treatment regimes and animal strains. In pups used in the renal function tests the teratogenic effects of chlorambucil were more severe and more prevalent than those produced by nitrofen. (The latter compound produced a high incidence of diaphragmatic hernias and, hence, affected pups generally died.) The teratogenic dose of chlorambucil affected a variety of renal functional indices, while the subteratogenic dose did not. Nitrofen affected renal function at all doses. It is noteworthy that a diminished concentrating response (in the absence of any significant change in morphological and biochemical parameters) was observed at the lowest dose level tested. While pups with overtly malformed kidneys performed quite poorly on the

function tests, pups with apparently normal-appearing kidneys also showed evidence of renal dysfunction. These results extend into the postnatal period earlier observations that exposure to chlorambucil and nitrofen during gestation retards the development of the kidney. The functional impairments of the kidneys observed for both compounds indicate that the tests utilized in this experiment are capable of detecting alterations in development in apparently morphologically normal offspring.

The findings from the mercuric chloride study were perhaps the most surprising of the entire experiment. Dose levels of mercuric chloride that produce acute renal failure in adult rats failed to alter any index of renal function in newborn rats. These results are contrary to those observed in the 5- to 30-d-old rabbits (Wold et al., 1977) but similar to those reported in neonatal rats exposed to mercuhydrin, an organomercurial diuretic (Wachstein and Robinson, 1965). The later authors found no evidence of histological damage to the kidneys until the rat pups reached 14 d of age. Other instances of resistance to chemically induced renal toxicity in young animals have been observed in cephaloridine-treated rabbits (Wold et al., 1977) and in gentamicin-treated dogs (Cowan et al., 1980). Wold et al. attributed the tolerance at younger ages to the incomplete development of the renal anionic transport system, which prevented toxic levels of cephaloridine from accumulating in the renal tubules, while Cowan et al. hypothesized that nephrons in the juxtamedullary cortex of neonatal puppies receive the major fraction of renal blood and that damage to those nephrons would be masked by successive development of more superficial nephrons as nephrogenesis proceeds. Nephrogenesis also proceeds in a centripetal fashion in the rat, and in fact, the rate of development is very similar in the puppy and the neonatal rat (Eisenbrandt and Phemister, 1979; Kittleson, 1917). Such a series of events (in which the normal developmental process "protects" against nephrotoxicity) could be responsible for the lack of toxicity that is observed for mercuric chloride. It should be noted, however, that other work on mercuric chloride nephrotoxicity (Bidani et al., 1979) indicated that young rats (i.e., 2 wk old) recovered more slowly from acute renal failure than did old rats (4 and 8 wk old). At present, it is not known what factors distinguish rabbit and rat renal development that could account for the differences in sensitivity of the very young animal to mercuric-chloride-induced nephrotoxicity, nor what affects organification of the mercury would have on its toxicity. Studies are currently being conducted in our laboratory to elucidate the developmental profile of mercuric chloride and to define mechanisms by which the pups may be protected from the toxic insult.

In summary, we have applied a series of renal function tests to neonatal rat exposed perinatally to suspected renal toxicants. For chorambucil, the effects on renal function proved equally as sensitive as effects on morphology. For nitrofen, however, the physiological responses

of the kidney to a period of fluid deprivation proved a more sensitive indicator of developmental toxicity than growth or morphological effects on that kidney. Finally, the neonatal rat kidney proved surprisingly resistant to mercuric-chloride-induced nephrotoxicity, providing evidence that young rodents are not always more sensitive to toxic insults than are adults.

REFERENCES

Allen, L. H. and Zeman, F. J. 1973. Kidney function in the progeny of protein-deficient rats. *J. Nutr.* 103:1467–1478.

Berndt, W. O. 1976. Renal function tests: What do they mean? A review of renal anatomy, biochemistry and physiology. *Environ. Health Perspect.* 15:55–71.

Bidani, A., Churchill, P. C., Fleischmann, L., and Becker-McKenna, B. 1979. $HgCl_2$-induced acute renal failure in the developing rat. *Pediatr. Res.* 14:183–186.

Braunlich, H., Fleck, C., Weise, C., and Stopp, M. 1979. Stimulation of kidney function in rats of different ages injured by nephrotoxic agents. *Exp. Pathol.* 17:486–492.

Costlow, R. D. and Manson, J. M. 1981. The target organ in the neonatal deaths induced by nitrofen (2,4,-dichlorophenyl-*p*-nitrophenyl ether). *Toxicology* 20:209–227.

Cowan, R. H., Jukkola, A. F., and Arant, B. S., Jr. 1980. Pathophysiologic evidence of gentamicin nephrotoxicity in neonatal puppies. *Pediatr. Res.* 14:1204–1211.

Eisenbrandt, D. L. and Phemister, R. D. 1979. Postnatal development of the canine kidney: Quantitative and qualitative morphology. *Am. J. Anat.* 154(2):179–193.

Gibson, J. E. 1976. Perinatal nephropathies. *Environ. Health Perspect.* 15:121–130.

Hall, S. M. and Zeman, F. J. 1968. Kidney function in the progeny of rats fed a low protein diet. *J. Nutr.* 95:49–54.

Johnson, D. R. and Kleinman, L. I. 1979. Effects of lead exposure on renal function in young rats. *Toxicol. Appl. Pharmacol.* 48:361–367.

Johnson, D. R., Kleinman, L. I., and Whitsett, J. A. 1980. Effect of lead on renal responsiveness to antidiuretic hormone in young rats. *Toxicol. Appl. Pharmacol.* 53:299–305.

Kavlock, R. J. and Gray, J. A. 1982. Evaluation of renal function in neonatal rats. *Biol. Neonate* 41:279–288.

Kavlock, R. J., Chernoff, N., Rogers, E., Whitehouse, D., Carver, B., Gray, J., and Robinson, K. 1982. An analysis of fetotoxicity using biochemical endpoints of organ differentiation. *Teratology* 26:183–194.

Kimbrough, R. D., Gaines, T. B., and Linder, R. E. 1974. 2,4-Dichlorophenyl-*p*-nitrophenyl ether (TOK). Effects on the lung maturation of rat fetus. *Arch. Environ. Health* 28:316–320.

Kittelson, J. A. 1917. The postnatal growth of the kidney of the albino rat, with observations on an adult human kidney. *Anat. Rec.* 13:385–408.

McDowell, E. M., Nagel, R. B., Zalme, R. C., McNeil, J. S., Flamenbaum, W., and Trump, B. F. 1976. Studies on the pathophysiology of acute renal failure. I. Correlation of ultrastructure and function in the proximal tubule of the rat following administration of mercuric chloride. *Virchows Arch. B Cell Pathol.* 22:173–196.

Monie, I. W. 1961. Chlorambucil induced abnormalities of the urogenital system of rat fetuses. *Anat. Rec.* 139:145–153.

Peterson, G. L. 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Anal. Biochem.* 83:346–356.

SAS. 1979. *SAS User's Guide, 1979 Edition.* Cary, N.C.: SAS Institute.

Wachstein, M. 1955. Histochemical staining reactions of the normal functioning and abnormal kidney. *J. Histochem.* 3:246–270.

Wachstein, M. and Robinson, M. 1965. Neonatal resistance to nephrotoxic renal tubular necrosis in the rat. *Fed. Proc.* 24:619.

Wold, J. S., Joost, R. R., and Owen, N. V. 1977. Nephrotoxicity of cephaloridine in newborn rabbits: Role of the renal transport system. *J. Pharmacol. Exp. Ther.* 201:778–785.

Woo, D. C. and Hoar, R. M. 1972. "Apparent hydronephrosis" as a normal aspect of renal development in late gestation of rats: The effect of methyl salicylate. *Teratology* 6:191–196.

Zeman, F. J. 1968. Effects of maternal protein restriction on the kidney of the newborn young of rats. *J. Nutr.* 94:111–116.

Zalme, R. C., McDowell, E. M., Nagle, R. B., McNeil, J. S., Flamenbaum, W., and Trump, B. F. 1976. Studies on the pathophysiology of acute renal failure. II. A histochemical study of the proximal tubule of the rat following administration of mercuric chloride. *Virchows Arch. B Cell Pathol.* 22:197–216.

Received April 17, 1982
Accepted August 3, 1982